

ATTACHMENT A

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: Danette Vanessa Choi

Filed: March 13, 2006

Group Art Unit: 1781

Confirmation No.: 9136

Serial No: 10/536,617

Examiner: Chawla, Jyoti

For: PAPAYA PUREE AND THE USE OF THE SAME

DECLARATION BY DR. CONCETTA GIULIANI
(UNDER 37 CFR § 1.132)

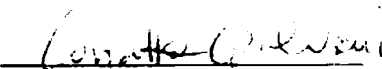
Commissioner for Patents
P. O. Box 1450
Alexandria, VA 22313-1450

1. My name is Dr. Concetta Giuliani. I am a Scientific Manager at Botanical Products International, F. M. Brenner GmbH, Hauptstrasse 10, 2392 Wienerwald/Grub, Austria.
2. I am an employee of Botanical Products International and receive no special benefits from the execution of this declaration.
3. I have obtained a Ph.D. degree in Plant Genetics and Molecular Biology from the University Of Vienna (Austria). A copy of my Curriculum Vitae is enclosed. (Attachment 1). My research interests are in the field of plant sciences and botanical products. I have research experience in plant proteins, including enzymes in different plant systems. In that respect, I am knowledgeable about the published literature and suitable experimental techniques in these areas. I am also knowledgeable about manufacturing method of CARICOL® papaya product, and analytical tests conducted throughout manufacture.
4. I am not an inventor of the above-identified patent application.

5. I have reviewed the above-identified U.S. patent application and understand that the claims of the above-referenced U.S. patent application are directed to a papaya puree preparation, methods for preparing a papaya puree preparation as well as administration of the preparation for achieving certain health benefits.
6. In that regard, I was asked by the Applicant's representative to explain a surprising and unexpected result obtained with respect to activity of papain, an important proteolytic enzyme, in the papaya puree preparation made according to the method disclosed in the present patent application.
7. As discussed in the present application, as of effective filing date of the present application (which I understand is November 26, 2002), it was well established in the field that papain is not heat resistant and is inactivated at temperatures between 60°C - 80°C. When subjected to temperatures above 85°C, papain is thermally inactivated, and thus becomes ineffective with respect to its enzyme activity (*see*, specification, page 5, 2nd full par.).
8. This point is underscored by the studies of Winnick *et al.* discussed below.
(Attachment 2: Winnick T. *et al.*, Physicochemical properties of the proteolytic enzyme from the latex of the milkweed, *Asclepias Speciosa Torr.* Some comparisons with other proteases. III. Kinetics of the heat inactivation of papain, bromelin, and asclepain: *J Gen Physiol.* (1940), 23(3): 301-308)
9. Winnick *et al.*, reported the kinetics of heat inactivation of papain at three different temperatures (75°C, 80°C and 83°C) and showed that at 83°C, papain is destroyed (inactivated) rapidly (<10 minutes) (*see*, Attachment 2-Winnick, p. 303, Fig.1).
10. Further, Winnick *et al.* note that heat inactivation of papain could not be reversed by cooling the solution (*see*, Attachment 2-Winnick, p. 301, abstract, 2nd par.)
11. During manufacture of a papaya puree prepared according to the method described in the present preparation (*e.g.*, CARICOL®), the preparation is cooked by heating up to a boiling temperature of approximately 212°F (100°C) and held at that temperature throughout the length of the cooking step. The cooking (boiling) step is preferably carried out for at least 2 hours (*see*, specification, paragraph spanning p. 2-3).

12. I attach herewith a report of a validated scientific assay, which shows that despite the lengthy boiling process (described above) papain activity of a CARICOL® sample did not undergo reduction. In fact, surprisingly, a 4-fold increment in papain activity of the CARICOL® sample (3105) was observed in comparison to a reference papaya puree preparation (3106) which was Pasteurized at 90°C for around 20 mins (*see, Attachment 3- Lefo Institute Examination report*).
13. Thus, in view of the knowledge in the field, as discussed in par. 8-11 above, as of the effective filing date of the present application, the result discussed above in par. 13 could not have been expected.
14. I conclude that the high level of papain activity observed in a papaya puree preparation (*e.g.*, CARICOL®) prepared according to the present method involving application of prolonged boiling heat is indeed very surprising to me.
15. I declare that the above statements are true to the best of my knowledge.

Date: 06/06/2011


Dr. Concetta Giuliani



BOTANICAL PRODUCTS INTERNATIONAL
P.M. Bremner GmbH
A-2392 Wienerwald Grub Hauptstrasse 10